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BPTF is essential for T cell homeostasis and function

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Abstract

Bromodomain PHD Finger Transcription Factor (BPTF), a ubiquitously expressed ATP-dependent chromatin-remodeling factor, is critical for epigenetically regulating DNA accessibility and gene expression. While BPTF is important for the development of thymocytes, its function in mature T cells remains largely unknown. By specifically deleting BPTF from late DN3/DN4 stage of developing T cells, we found that BPTF was critical for the homeostasis of T cells via a cell intrinsic manner. In addition, BPTF was essential for the maintenance and function of Treg cells. Treg cell-specific BPTF deletion led to reduced Foxp3 expression, increased lymphocyte infiltration in the non-lymphoid organs and a systemic autoimmune syndrome. These findings therefore reveal a vital role for BPTF in T and Treg cell function and immune homeostasis.

Keywords

BPTF; T cell homeostasis; Treg cell; Foxp3; autoimmunity

INTRODUCTION

In eukaryotes, genomic DNA is wound around histone complexes to form nucleosomes (1–3). Nucleosomes further organized into more compact structures of chromatins and chromosomes in the nucleus (4). The highly compact chromatin structure makes the genetic loci inaccessible to factors controlling gene expression and DNA replication. Therefore, remodeling chromatin structure into an open configuration is a pre-requisite for gene transcription and DNA replication to allow regulatory factors to access DNA (5, 6).

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Chromatin-remodeling complexes are critical cellular factors reconfiguring chromatin structure to epigenetically regulate DNA accessibility and gene expression. The central subunit of chromatin-remodeling complex is an ATPase that hydrolyzes ATP to acquire the energy needed to loosen condensed chromatin structure (7). Four families of ATPase-dependent chromatin-remodeling complexes have been characterized to date: SWI/SNF family (switching defective/sucrose non-fermenting), ISWI (imitation SWI) family, NURD (nucleosome remodeling and deacetylation)/Mi-2/CHD (chromodomain, helicase, DNA binding) family and INO80 (inositol requiring 80) family (7). These chromatin remodelers control a myriad of biological processes including cell growth, proliferation, survival, differentiation and function in a cell type-specific manner (2, 8, 9). The deregulation of chromatin remodeling complexes often leads to debilitating and fatal diseases including developmental deformity, inflammatory disease (10) and cancer (11). Therefore, in order to understand disease etiology and devise effective therapies, it is important to reveal how chromatin-remodeling complexes control the functions of a specific cell type. While great strides have been made to uncover their roles in tumor cells (12, 13), much less is known about how the chromatin-remodeling complexes in controlling T cell functions.

T cells play pivotal roles in immunity by eliciting antigen specific response, establishing immunological memory, and directing different types of immunity including cytotoxic, type 1 and type 2 responses (14–17). In addition, Foxp3-expressing regulatory T (Treg) cells are essential for immune suppression and self-tolerance. Defective T cell function often leads to increased susceptibility of infection and cancer development (18). Yet, over-exuberant T cell function contributes to autoimmune and inflammatory disease. Chromatin remodeling complexes are required for thymic T cell development (19) by integrating signaling from TCR and co-stimulatory molecules (20, 21). In particular, Brg, the ATPase subunit of BAF complex, is critical for the development of double negative thymic T cells and Th1/Th2 cell differentiation (22–24). Nonetheless, Brg is largely dispensable for the homeostasis of mature T cell (19). These findings suggest that chromatin-remodeling complexes, such as Brg containing BAF complex, control T cell function in a cell-type specific manner. More importantly, it raises a question of whether the four known chromatin-remodeling complexes can compensate for each other and play redundant roles in mature T cells. To address this question, we investigated whether and how bromodomain PHD finger transcription factor (BPTF), an integral component of nucleosome remodeling factor (NURF) chromatin remodeling complex, is involved in mature T cell function.

BPTF is a 3,046 aa protein containing histone or DNA binding motifs (25, 26). It is ubiquitously expressed and the largest component of the nucleosome remodeling factor (NURF) (27). BPTF binds to nucleosomes with trimethylated lysine 4 on histone H3 and acetylated histone, where NURF complexes remodel chromatin to regulate gene expression in a locus specific manner (26). BPTF, a central component of NURF complexes, is required for the development of the early embryo (28) and thymocytes (27). Nevertheless, whether and how BPTF controls mature T cell homeostasis and function remain unexplored.

Here, by specifically deleting BPTF from late DN3/DN4 stage of developing T cells, we found that BPTF was vital for the homeostasis of T cells. In addition, the thymic development of Treg cells required BPTF. Further investigation revealed that BPTF was

essential for the function of mature Treg cells in the periphery. Deletion of BPTF specifically in Foxp3-expressing Treg cells led to defective suppressive function of Treg cells, unstable Foxp3 expression, and an inflammatory syndrome in mice. These findings therefore highlighted an essential role for BPTF in T and Treg cell function and immune homeostasis.

Materials and Methods

Mice

Cd4Cre, *FGC*, *FGC-tdtomato*, *Bptf*$^{fl/fl}$, *Rag1*$^{-/-}$ and CD45.1 congenic wild-type mice were on the C57BL/6 background. All mice were housed and bred in specific pathogen-free conditions in the animal facility at the University of North Carolina at Chapel Hill. All mouse experiments were approved by Institution Animal Care and Use Committee of the University of North Carolina.

Mixed-bone marrow chimeras

Bone marrow cells were isolated from the femur bones of sex- and age-matched *Cd4Cre;Bptf*$^{fl/fl}$ (CD45.2^{+}) mice and wild-type (CD45.1.2^{+}) mice or *FGC:Bptf*$^{fl/wt}$ (CD45.1.2) and *FGC:Bptf*$^{fl/fl}$ (CD45.2.2). Bone marrow cells (5×10^6) from each donor were mixed and transferred into irradiated *Rag1*$^{-/-}$ or C57BL/6 recipient mice (CD45.1^{+}). T cell populations of each donor were detected in the recipients 10–12 weeks after transfer.

Quantitative RT-PCR

Total RNA was extracted from T cells with TRIzol reagent according to the manufacturer's instructions (Invitrogen) and was reverse-transcribed into c-DNA with Superscript III reverse transcriptase (Bio-Rad). Quantitative PCR was performed on QuantStudio® 6 Flex Real-Time PCR System. Primers for *Bptf*: forward: 5′-GCAGCTTCAGGAGCCATAGTAC-3′; reverse: 5′-GGAGAACGAGGCCGATGTAC-3′; *Hprt*: forward: GGGGGCTATAAGTTCTTTGC; reverse: 5′-TCCAACACTTCGAGAGGTCC-3′.

Cell Proliferation and Suppression Assay

CD4^{+}GFP^{+} Treg cells from *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice and CD4^{+}CD25^{-}CD45RB^{high} responder T cells from WT (CD45.1) mice were sorted on a FACSAria III (BD Biosciences) in the UNC Flow Cytometry Core Facility. To assess the efficacy of Treg cell-mediated immune suppression *in vitro*, 1×10^5 sorted responder T cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and mixed with different amounts of Treg cells (as indicated). Cell mixtures were then stimulated with soluble CD3 antibody (1 μg/ml) in the presence of 5×10^5 irradiated (3000 cGy) T cell-depleted splenocytes as APC. The proliferation of responder cells was assessed at 72 hr post-stimulation on FACSCanto (BD Biosciences) in the Lineberger Comprehensive Cancer Center Human Immunology Core.

Flow Cytometry and Cell Sorting

Lymphocytes were isolated from the various organs of age- and sex-matched mice of 8–16 weeks of age. Fluorescence-conjugated anti-CD4 (GK1.5), anti-CD8 (53–6.7), anti-CD25 (PC61.5), anti-CD44 (IM7), anti-CD69 (H1.2F3), anti-CD62L (MEL-14), anti-PD-1 (RMP1-30), anti-CD45.1 (A20), anti-CD45.2 (104), and anti-IFN-γ (XMG1.2), anti-IL-4 (11B11) (eBioscience), and anti-IL17A (TC11-18H10.1) (Biolegend), and CTLA4 (BNI3, BD Bioscience) were purchased. Surface and intracellular staining were performed as manufacturer's protocols. Stained cells were analyzed on a LSRII (BD Biosciences) in the UNC Flow Cytometry Core Facility or FACSCanto (BD Biosciences) in the Lineberger Comprehensive Cancer Center Human Immunology Core.

Histology

Different tissues from 4–5 mice of *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice at 5–7 months age were harvested and immersed in 10% Shandon Formal Fixx (Thermo Fisher) at room temperature for 2 days and then were paraffin-embedded. Histologic sections (5 μm) were stained with H&E and were evaluated visually under microscopy.

Statistical analysis

Data from at least three sets of samples were used for statistical analysis. Statistical significance was calculated by Student's *t*-test. A p value < 0.05 was considered to be statistically significant.

RESULTS

T cell thymic development in *Cd4Cre;Bptf*$^{fl/fl}$ mice

To study the function of BPTF during T cell development, we crossed *Cd4Cre* mice (29) with *Bptf*$^{fl/fl}$ mice (28) to generate *Cd4Cre;Bptf*$^{fl/fl}$ mice. *Bptf* gene was found deleted in the $CD4^+CD8^+$ double-positive (DP) thymocytes (supplemental Fig. S1A). The population of DN (double negative) thymocyte were largely normal in *Cd4Cre;Bptf*$^{fl/fl}$ mice (supplemental Fig. S1B). The numbers of thymocytes were similar between wild-type (WT) and *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 1A). The number and distribution of CD4SP (single positive) and CD8SP thymocytes were largely comparable between *Cd4Cre;Bptf*$^{fl/fl}$ and *Cd4Cre;Bptf*$^{fl/wt}$ mice (Fig. 1A and B). In addition, the expression of thymocyte maturation markers including CD44, CD69 and CD24 appeared normal in CD4SP and CD8SP thymocytes in the absence of BPTF (Fig. 1C and D). Nonetheless, we noticed that Foxp3 expressing CD4SP nTreg cells was significantly reduced in the thymus of *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 1E) with normal expression of CD25 and CD44 (supplemental Fig. S1C). Therefore, in *Cd4Cre;Bptf*$^{fl/fl}$ mice, the T cell thymic development appeared largely normal, while the Treg cell thymic generation was defective.

BPTF is critical for T cell homeostasis in the periphery

We further investigated whether BPTF deletion affected the homeostasis of mature T cells in the periphery. The percentages and numbers of $CD4^+$ and $CD8^+$ T cells in the peripheral lymph nodes (pLNs) and spleens of *Cd4Cre;Bptf*$^{fl/fl}$ mice were found approximately 6-fold

and 5-fold lower than those of *Cd4Cre;Bptf*$^{fl/wt}$ mice and B cell number was increased by about 20% (Fig. 2A, 2B and supplemental Fig. S2A). In addition, CD4$^+$ T cells from *Cd4Cre;Bptf*$^{fl/fl}$ mice displayed an activated phenotype, showing an increased proportion of CD44^{high}CD62^{low} effector T cells in the PLNs and spleens (Fig. 2C). In agreement with this observation, a larger portion of CD4$^+$ T cells from *Cd4Cre;Bptf*$^{fl/fl}$ mice produced interferon-γ (IFN-γ) but most pronounced was the profound elevation of IL-17 compared to their counterpart cells from *Cd4Cre;Bptf*$^{fl/wt}$ mice (Fig. 2D). CD8$^+$ T cells displayed activated phenotype (Fig. 2C) with highly elevated IFN-γ production in *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 2D).

Treg cells are critical to suppress T cell activation in the peripheral. We found that the production of Treg cells was reduced in the thymus of *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 1D). However, the distribution of Treg cells appeared normal in the pLNs and spleen of *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 2E).

The T cell number in the periphery was greatly reduced, causing a lymphopenic environment in the *Cd4Cre;Bptf*$^{fl/fl}$ mice. Such an environment may lead to aberrant T cell distributions (30). In order to investigate if the observed defect associated with BPTF deletion is due to cell-intrinsic effects, we generated mixed-bone marrow (BM) chimeras by reconstituting *Rag1*$^{-/-}$ mice with equal numbers of BM cells isolated from *Cd4Cre;Bptf*$^{fl/fl}$ mice (CD45.2$^+$) and *Cd4Cre;Bptf*$^{fl/wt}$ mice (CD45.1$^+$CD45.2$^+$). Compared to co-existing *Cd4Cre;Bptf*$^{fl/wt}$ T cells, the number of *Cd4Cre;Bptf*$^{fl/fl}$ SP thymocytes was decreased (Fig. 2F). Strikingly, BPTF deletion led to a complete absence of CD4$^+$ and CD8$^+$ T cells in the periphery (Fig. 2F). These findings suggest that, while BPTF contributes to the thymocyte development, it is absolutely required for the homeostasis of CD4$^+$ and CD8$^+$ T in the periphery. Indeed, further analysis revealed that *Bptf* alleles were incompletely deleted and substantial amounts of *Bptf* mRNA were detected in the periphery of CD4$^+$ and CD8$^+$ T cells isolated from *Cd4Cre;Bptf*$^{fl/fl}$ mice (supplemental Fig. S2B and data was not shown), suggesting “escapee” T cells existed in the periphery of these mice.

Treg-specific deletion of BPTF leads to autoimmunity

We found the number of Foxp3-expressing thymic Treg cells was reduced in *Cd4Cre;Bptf*$^{fl/fl}$ mice (Fig. 1) suggesting that BPTF is important for the thymic generation of Treg cells. This observation promoted us to ponder if BPTF plays a role in Treg cell function in the periphery. The failure of generating BPTF-deficient T cells in *Cd4Cre;Bptf*$^{fl/fl}$ prevented us from analyzing Treg cells in these mice. To address this question, we generated Treg cell-specific BPTF-deficient mice by crossing *Bptf*$^{fl/fl}$ mice with *Foxp3-EGFP-cre* mice (31), hereafter referred to as *FGC* mice. FGC mice bear a BAC transgene expressing enhance green fluorescence protein (EGFP) and Cre recombinase under the control of Foxp3 promoter. In *FGC* mice, EGFP expression reliably marks Foxp3-expressing Treg cells, and Cre-mediated gene deletion occurs specifically in Foxp3-expressing Treg cells (supplemental Fig. S3A). The distribution of T cells in the thymus, spleen and pLN appeared comparable between *FGC:Bptf*$^{fl/fl}$ and *FGC:Bptf*$^{fl/wt}$ mice (Fig. 3A and supplemental Fig. S3B). Nevertheless, peripheral non-Treg CD4$^+$ and CD8$^+$ T cells displayed activated phenotype (CD44^{high}CD62^{low}) (supplemental Fig. S3C and S3D) with elevated IFN-γ

production in the *FGC:Bptf*$^{fl/fl}$ mice (Fig. 3B and 3C). Importantly, we found that *FGC:Bptf*$^{fl/fl}$ mice developed an autoimmune syndrome by 5–7 months of age with lymphocytic infiltration into non-lymphoid organs, including the lung and kidney (Fig. 3D). The numbers of infiltrated T cells in the relative immune privilege organs including ovary was also significantly increased in *FGC:Bptf*$^{fl/fl}$ mice (Fig. 3E) with elevated IFN-γ production (Fig. 3F). Therefore, Treg cell specific BPTF deletion led to aberrant T cell activation and autoimmune syndrome in mice.

BPTF is required for Treg cell homeostasis

The T cell activation and autoimmunity in *FGC:Bptf*$^{fl/fl}$ mice suggested a defect in Treg cell population in these mice. Indeed, Foxp3-expressing (GFP$^+$) Treg cell population was significant reduced in the spleen and PLNs of *FGC:Bptf*$^{fl/fl}$ mice (Fig. 4A). Foxp3$^+$ T cells could convert to Foxp3$^-$ (exFoxp3) T cells (32). To investigate if Treg cells down regulate Foxp3 expression in the absence of BPTF, we crossed *FGC:Bptf*$^{fl/fl}$ mice with the ROSA26-loxP-STOP-tdtomato reporter strain to obtain Foxp3 fate mapping mice (referred as *FGC:Bptf*$^{fl/fl}$*:tdtomato* mice). We found the percentages of ex-Foxp3 Treg cells increased in the pLN and spleen of *FGC:Bptf*$^{fl/fl}$*:tdtomato* mice when compared to those of *FGC:Bptf*$^{fl/wt}$*:tdtomato* mice (Fig. 4B and supplemental Fig. S3E). It suggests BPTF is required to maintain Foxp3 expression. While BPTF-deficient Foxp3$^+$ Treg cells in *FGC:Bptf*$^{fl/fl}$ mice expressed normal levels of PD-1, CTLA-4 and increase levels of CD25 (Fig. 4C), they showed reduced suppressive activities (Fig. 4D). Taken together, Treg cell-specific BPTF deletion led to unstable Foxp3 expression and impaired suppressive function of Treg cells.

The effect of BPTF deletion on Treg cells is cell intrinsic

To exclude the possibility that the effects of BPTF deletion in Treg cells were due to the cell-extrinsic inflammatory environment observed in *FGC:Bptf*$^{fl/fl}$ mice, we generated mixed-bone marrow chimeras by adoptive transfer of equal number of wild-type (CD45.1$^+$CD45.2$^+$) and *FGC:Bptf*$^{fl/fl}$ BM cells (CD45.2$^+$) into lethally irradiated C57BL/6 mice (CD45.1$^+$). In the fully reconstituted mixed chimeras mice, the numbers of *FGC:Bptf*$^{fl/fl}$ Treg cells was much less than those of *FGC:Bptf*$^{fl/wt}$ Treg cells in the peripheral and the thymus of the chimeric mice (Fig. 5A and 5B). The non-Treg cells were not apparently activated in the reconstituted chimeric mice (supplemental Fig. S4). In agreement with this finding, IFN-γ production by CD4$^+$ and CD8$^+$ T cells was normal in reconstituted chimeric mice (Fig. 5C). These findings suggest that the activated phenotype of non-Treg cells in the *FGC:Bptf*$^{fl/fl}$ mice was due to a defect in BPTF Treg cells, a phenotype that could be rescued by the presence of wild-type Treg cells. By analyzing the phenotypes of Treg cells in the reconstituted chimeric mice, we found that the CTLA-4 and PD-1 expression was slightly lower in BPTF deficient Treg cells than in wild-type Treg cells (Fig. 5D). These results therefore demonstrated that BPTF controls Treg cell function through a cell-intrinsic mechanism.

DISCUSSION

Treg cells play a critical role in immune homeostasis and self-tolerance (33, 34). What factors control the development and maintenance of Treg cell has been under intensive investigation. The current study shows that BPTF, an ATP-dependent chromatin-remodeling factor, was required for the development and maintenance of Treg cells in the periphery. Lack of BPTF in Treg cells perturbed the homeostasis and functions of Treg cells and Foxp3 expression, ultimately leading to aberrant immune activation and an autoimmune syndrome. Therefore, BPTF is central for self-tolerance and immune homeostasis by being required for stabilizing Treg function and Foxp3 expression.

Several studies suggested that BPTF is needed for the development of mesoderm, endoderm in early stage of mouse embryo. BPTF, like Brg-1, is required for the maturation of CD4/CD8 TCRβ^+ thymocytes by using Lck-cre mediated deletion during DN stage of thymocyte development (27). These findings demonstrate that both BPTF and Brg-1 have critical and non-redundant function to control early thymocyte development. To study whether these factors are important for T cell function after DN stage, CD4-Cre mediated deletion can be used. Previous study has revealed that Brg-1 was largely dispensable for T cell function after DN stage (19), suggesting that chromatin remodel complex controls T cell function in a cell type specific manner. It is therefore an interesting question as whether BPTF is important for T cell function after DN stage. In the current study, by deleting BPTF starting from the late DN3/DN4 stage using CD4-Cre, we found that BPTF, unlike Brg-1, was important for late T cell development and essential for the peripheral maintenance of mature T cells. Therefore, BPTF and Brg-1 control T cell function in a non-overlapping, cell type specific fashion. This notion is further supported by the finding that, compared to Treg cell-specific Brg-1 deletion, BPTF deletion in the Treg cells led to much more severe Treg functional defect and autoimmune manifestation (19). In addition, we found that BPTF was critical for the Treg cell development in the thymus of *Cd4Cre:Bptf*$^{fl/fl}$ mice. BPTF deletion post Foxp3 expression led to unstable Foxp3 expression in *FGC:Bptf*$^{fl/fl}$ mice (Fig. 4B). These findings collectively argue that, chromatin remodeling complexes function in a cell type specific manner. The molecular and epigenetic mechanisms underlying such observations therefore warrant future investigation.

Previous studies have shown that Treg cells may lose Foxp3 expression and turn into ‘exFoxp3’ cells (35–37). This can occur in inflammatory environment such as MOG-induced EAE, SLE or diabetes (38–40). Here we found that BPTF is required for the stability of Foxp3 expression. BPTF deletion led to increased portion of exFoxp3 T cells. Foxp3 expression is epigenetically regulated by both histone and DNA epigenetic modifications (41). H3K4me3 has been associated with Foxp3 promoter and CNS(s) in Treg cells (42). BPTF contains a bromodomain-proximal PHD finger and is involved in the formation of protein complexes recognizing H3K4me3 (25, 43). In light of the current finding that BPTF is important for Foxp3 expression and Treg cell function, it is reasonable to believe that BPTF controls Treg cell function via an epigenetic mechanism, a notion to be validated in the future.

Dysregulated function of chromatin remodeling complexes often leads to fatal diseases including autoimmune and cancers (44, 45). Interestingly, the defect of Treg cells in *FGC:Bptf*$^{fl/fl}$ mice contributed to aberrant T cell activation and autoimmunity in adult mice. While T cells in the periphery of *Cd4Cre:Bptf*$^{fl/fl}$ mice displayed an activated phenotype, it was likely due to a cell-extrinsic effect since substantial BPTF expression was detected in recovered peripheral T cells from *Cd4Cre:Bptf*$^{fl/fl}$ mice were. Because we could not obtain BPTF deficient mature T cells from *Cd4Cre:Bptf*$^{fl/fl}$ mice, we are not able to directly assess the function of BPTF deficient T cells. BPTF is required for MAPK and PI3K/AKT signaling and the survival of lung cancer cells (46). BPTF is also important for cell proliferation through interaction with c-Myc in fibroblasts and melanoma cells (47, 48). Since MAPK, PI3K/AKT and c-Myc dependent pathways can regulate T cell function (49–51), it is plausible that one or more of these pathway(s) is perturbed in the absence of BPTF in T cells.

Our study demonstrates a critical role for BPTF in the homeostasis and function of T and Treg cells and for immune homeostasis and self-tolerance. It highlights a central function for chromatin-remodeling complexes in T cell function, underscoring a potential of treating immune diseases by targeting chromatin-remodeling complexes in T cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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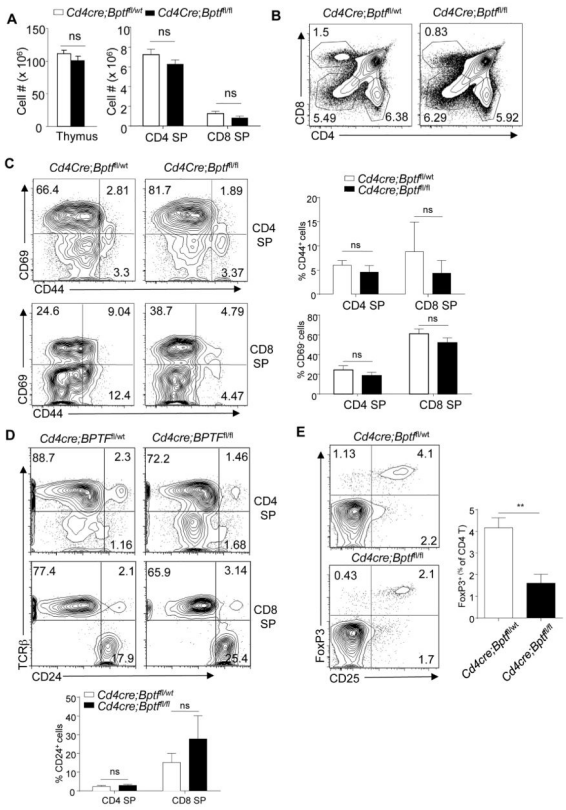

Fig. 1. T cell thymic development in *Cd4Cre;Bptf*$^{fl/fl}$ mice

A. Cell numbers of total, CD4SP, and CD8SP cells in the thymus of *Cd4Cre;Bptf*$^{fl/wt}$ and *Cd4Cre;Bptf*$^{fl/fl}$ mice. Means ± SD of three mice are shown.

B. The distribution of various thymocyte populations in the thymus of *Cd4Cre;Bptf*$^{fl/wt}$ and *Cd4Cre;Bptf*$^{fl/fl}$ mice were analyzed by flow-cytometry. Results are representation of three experiments.

C. Flow-cytometry of CD44 and CD69 expression on CD4SP and CD8SP thymocytes. Results are representative of three experiments.

D. The expression of CD24 and TCRβ on CD4SP and CD8SP thymocytes detected by flow-cytometry. Results are representative of three experiments.

E. Detection of Foxp3 expressing CD4SP thymocytes by flow-cytometry. Flow-cytometric results are representative of three experiments. Means ± SD of results from three mice are shown. NS, not significant; **P < 0.01.

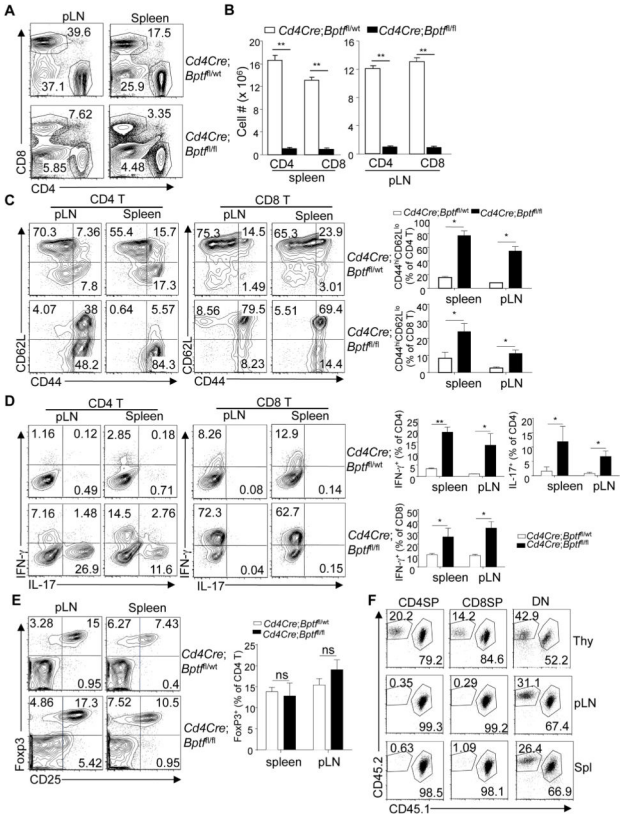

Fig. 2. BPTF is critical for T cell homeostasis in the periphery

A. CD4$^+$ and CD8$^+$ T cell population in pLN and spleen of *Cd4Cre;Bptf*$^{fl/wt}$ and *Cd4Cre;Bptf*$^{fl/fl}$ mice, assessed by flow-cytometry.

B. The comparison of the numbers of CD4$^+$ T and CD8$^+$ T cells recovered from *Cd4Cre;Bptf*$^{fl/wt}$ and *Cd4Cre;Bptf*$^{fl/fl}$ mice. Means ± SD of indicated T cell populations in three mice are shown.

C. The expression of CD44 and CD62L on CD4$^+$ and CD8$^+$ T cells was assessed by flow-cytometry. Means ± SD of indicated T cell populations in three mice are shown.

D. IFN-γ and IL-17 cytokine production in CD4 and CD8 T cells was assessed by flow-cytometry at 4hr after stimulation with PMA/ionomycin in the presence of BFA. Means ± SD of indicated T cell populations in three mice are shown.

E. Foxp3 expressing CD4$^+$ Treg cells in the pLN and spleen were detected by flow-cytometry. Means ± SD of indicated T cell populations in three mice are shown.

F. Mixed bone marrow chimera were created by transferring equal numbers of bone marrow cells from *Cd4Cre;Bptf*$^{fl/wt}$ (CD45.1.2$^+$) and *Cd4Cre;Bptf*$^{fl/fl}$ (CD45.2.2$^+$) mice into sub-lethally irradiated *Rag1*$^{-/-}$ mice. 10 to 12 weeks after transfer, the distribution of cells with different genotypes in the recipient mice were determined by flow-cytometry. All the results of flow-cytometry are representative of at least three experiments.

NS, not significant; *P < 0.05; **P < 0.01.

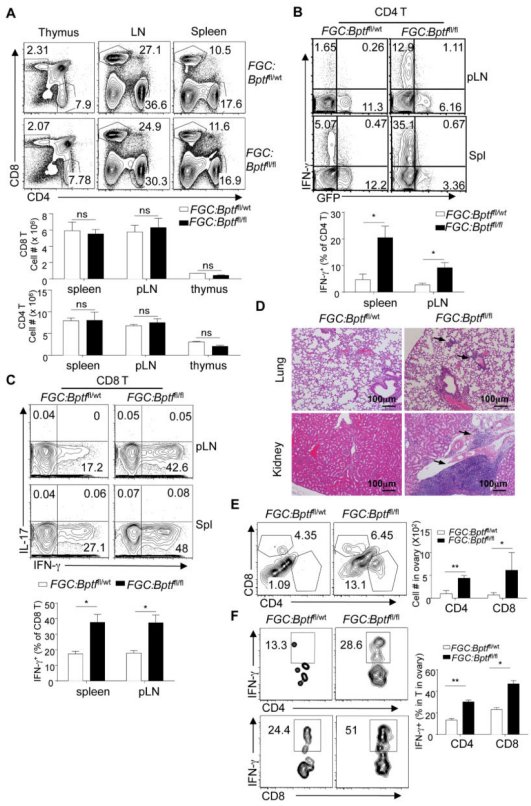

Fig. 3. Treg-specific deletion of BPTF leads to autoimmunity

A. $CD4^+$ T and $CD8^+$ T cells in pLN, spleen and thymus of *FGC:Bptf*$^{fl/wt}$ mice were detected by flow-cytometry. Results are representative of at least three experiments. The cell numbers was also counted. Means ± SD of three mice are shown.

B and **C**. IFN-γ producing CD4 (**B**) and CD8 (**C**) T cells were detected by flow-cytometry after stimulated with PMA/ionomycin in the presence of BFA. Results are representative of three experiments. The percentages of IFN-γ producing CD4 T and CD8 T cells in *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice were compared. Means ± SD of three mice are shown.

D. Histological analysis of lymphocytic infiltration in the lung and kidney of indicated mice by H&E staining.

E. Lymphocytes in the ovary of *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice were isolated and $CD4^+$ T and $CD8^+$ T cells were detected by flow-cytometry. The numbers of infiltrated T cells were counted. Results are representative of at least three experiments. Means ± SD of three mice are shown.

F. IFN-γ producing CD4 and CD8 T cells in the ovary of *FGC:Bptf*$^{fl/fl}$ mice were detected by flow-cytometry. Results are representative of three experiments. Means ± SD of three mice are shown.

NS, not significant; *P < 0.05; **P < 0.01.

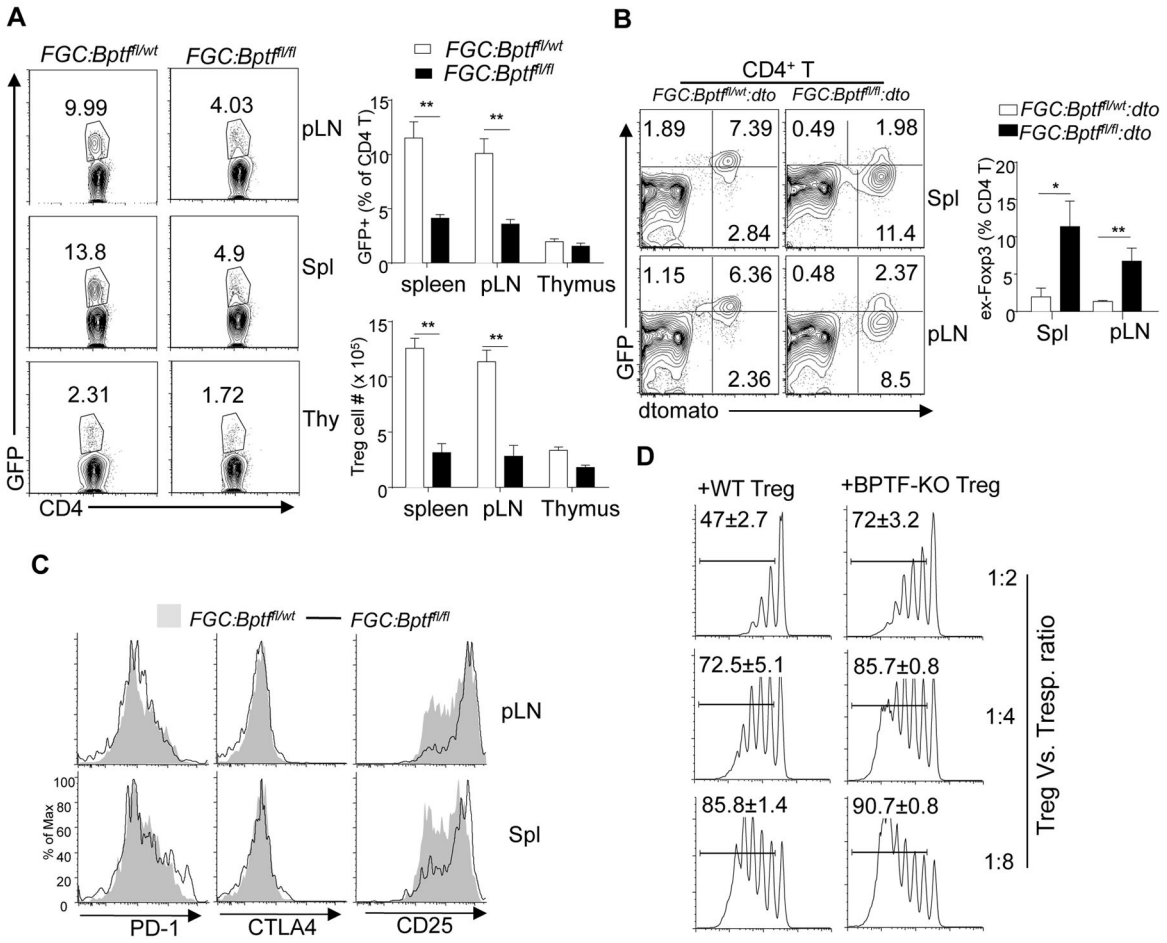

Fig. 4. BPTF is required for Treg cell homeostasis

A. The Foxp3-expressing (GFP$^+$) CD4 T cells were detected in *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice were flow cytometry. The percentages and the numbers of GFP$^+$ Treg cells was determined and compared. Means ± SD of three mice are shown.

B. The co-expression of GFP and dtomato in CD4 T cells isolated from *FGC:Bptf*$^{fl/wt}$:dto and *FGC:Bptf*$^{fl/fl}$:dto mice was assessed by flow-cytometry. The percentages of GFP$^-$dtomato$^+$ ex-Foxp3 Treg cells in CD4 T cells was determined and compared between *FGC:Bptf*$^{fl/wt}$:dto and *FGC:Bptf*$^{fl/fl}$:dto mice. Means ± SD of three mice are shown.

C. CD25, CTLA4 and PD-1 expression on the GFP$^+$ Treg cells isolated from *FGC:Bptf*$^{fl/wt}$ and *FGC:Bptf*$^{fl/fl}$ mice were detected and compared by flow-cytometry.

D. GFP$^+$ Treg cells sorted from *FGC:Bptf*$^{fl/wt}$ (WT) and *FGC:Bptf*$^{fl/fl}$ (BPTF-KO) mice were mixed with CFSE labeled, CD4$^+$CD25$^-$CD45RB^{high} responder T cells (Tresp.) sorted from wild-type C57BL/6 mice at indicated ratios. The proliferation of Tresp. cells was determined by CFSE dilution assessed by flow-cytometry 72 hours after activation. Results are representative of two experiments.

All the results of flow-cytometry are representative of at least three experiments unless stated otherwise.

*P < 0.05; **P < 0.01.

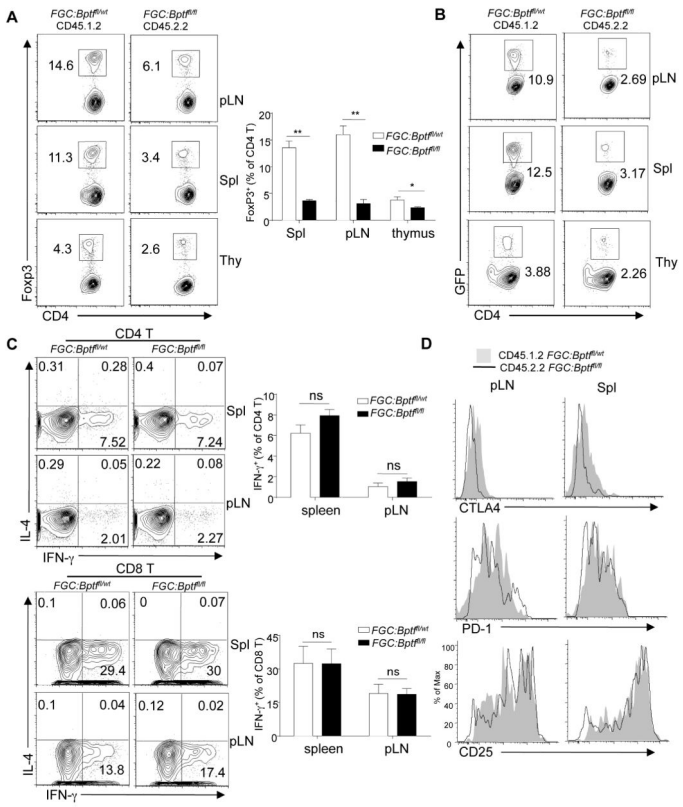

Fig. 5. The effect of BPTF deletion on Treg cells is cell intrinsic

Mixed bone marrow chimera was created by transferring equal numbers of bone marrow cells from *FGC:Bptf*$^{fl/wt}$ mice (CD45.1^{+}CD45.2^{+}) and *FGC:Bptf*$^{fl/fl}$ mice (CD45.2^{+}) mice into sub-lethally irradiated WT C57BL/6 mice (CD45.1^{+}).

A and **B**. Foxp3^{+} (**A**) and GFP^{+} (**B**) Treg cells from different donors were detected by flow cytometry. The percentages of Foxp3^{+} Treg cells in CD4 T cells of different origins in the recipient mice were determined. Means ± SD of three mice are shown.

C. IFN-γ and IL-4 expressing CD4^{+} and CD8^{+} T cells were detected by flow-cytometry. The percentages of IFN-γ expressing CD4 and CD8 T cells were determined. Means ± SD of three mice are shown.

D. The expression of CD25, CTLA4 and PD-1 on Foxp3^{+} Treg cells of different origins was assessed by flow-cytometry and compared.

All the flow-cytometry results are representative of at three experiments.

NS, not significant; *P < 0.05; **P < 0.01.